

Improved Method for Hexachlorobenzene and Mirex
Determination with Hexachlorobenzene
Confirmation in Adipose Tissue
Collaborative Study

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1980



TECHNICAL REPORT DATA			MS-79-064
(Please read instructions on the reverse before completing)			
1. REPORT NO. EPA-600/J-80-261	2. JOURNAL ARTICLE	3. PB81-151575	
4. TITLE AND SUBTITLE Improved Method for Hexachlorobenzene and Mirex Determination with Hexachlorobenzene Confirmation in Adipose Tissue: Collaborative Study		5. REPORT DATE April 1981	
7. AUTHOR(S) Randall R. Watts, David W. Hodgson, Howard L. Crist, and Robert F. Moseman		6. PERFORMING ORGANIZATION CODE	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Analytical Chemistry Branch Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711		8. PERFORMING ORGANIZATION REPORT NO.	
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development RTP 27711 Health Effects Research Lab US Environmental Protection Agency		10. PROGRAM ELEMENT NO. ABZE1A	
		11. CONTRACT/GRANT NO.	
		13. TYPE OF REPORT AND PERIOD COVERED	
		14. SPONSORING AGENCY CODE EPA-600/11	
15. SUPPLEMENTARY NOTES Published In: Journal of Official Analytical Chemists 63(5): 1128-1134, 1980			
16. ABSTRACT A previously published method for determination and confirmation of hexachlorobenzene (HCB) in adipose tissue was also applied to mirex residues was collaboratively studied by 12 laboratories. The procedure specifies direct application of an extracted or rendered fat sample to a Florisil cleanup column and one-fraction elution. Mirex and HCB are determined by direct GLC of the concentrated eluate. HCB residues are then confirmed by reaction with isopropanol to form the disubstituted bis-isopropoxy-tetrachlorobenzene (BITB) derivative. Mirex residues are destroyed by this reaction. All participants were asked to analyze an unknown standard mixture of HCB and mirex and 10 rendered chicken fat samples consisting of one blank and 9 samples fortified with unknown amounts of HCB and mirex. The 9 fortified samples represented 3 samples at each of 3 fortification levels. The HCB fortifications of 20.0, 33.3, and 50.0 ppb yielded average interlaboratory recoveries of 89.6, 87.4, and 92.6%, respectively. The respective coefficients of variation (CV) for HCB results were 9.1, 6.8, and 10.0%. The mirex fortifications of 150, 300, and 500 ppb yielded average interlaboratory recoveries of 89.0, 90.2, and 92.3%, respectively. The respective CV values for mirex results were 7.6, 16.5, and 18.1%. The method has been adopted official first action.			
17. KEY WORDS AND DOCUMENT ANALYSIS			
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group	
PRODUCT OF: NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161			
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC		19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES
		20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

Improved Method for Hexachlorobenzene and Mirex Determination with Hexachlorobenzene Confirmation in Adipose Tissue: Collaborative Study

RANDALL R. WATTS, DAVID W. HODGSON, HOWARD L. CRIST,
and ROBERT F. MOSEMAN

*Environmental Protection Agency, Health Effects Research Laboratory,
Research Triangle Park, NC 27711*

Collaborators: A. Barquet; J. Cain; J. Colcolough, Jr; V. Cox; H. Enos;
A. B. Filonow; B. Gardner; E. Gomes; H. Hetzler; R. A. Leavitt;
A.-M. C. Leventis; C. Morgade; R. Morgner; E. Richardson;
E. Slach; R. Stringer; J. Tessari; S. L. Warnick, R. L. Welch

A previously published method for determination and confirmation of hexachlorobenzene (HCB) in adipose tissue was also applied to mirex residues. A modified procedure for both residues was collaboratively studied by 12 laboratories. The procedure specifies direct application of an extracted or rendered fat sample to a Florisil cleanup column and one-fraction elution. Mirex and HCB are determined by direct GLC of the concentrated eluate. HCB residues are then confirmed by reaction with isopropanol to form the disubstituted bis-isopropoxytetrachlorobenzene (BITB) derivative. Mirex residues are destroyed by this reaction. All participants were asked to analyze an unknown standard mixture of HCB and mirex and 10 rendered chicken fat samples consisting of one blank and 9 samples fortified with unknown amounts of HCB and mirex. The 9 fortified samples represented 3 samples at each of 3 fortification levels. The HCB fortifications of 20.0, 33.3, and 50.0 ppb yielded average interlaboratory recoveries of 89.6, 87.4, and 92.6%, respectively. The respective coefficients of variation (CV) for HCB results were 9.1, 6.8, and 10.0%. The mirex fortifications of 150, 300, and 500 ppb yielded average interlaboratory recoveries of 89.0, 90.2, and 92.3%, respectively. The respective CV values for mirex results were 7.6, 16.5, and 18.1%. The method has been adopted official first action.

An investigation was conducted to select and collaboratively study a method suitable for determination of low levels of hexachlorobenzene (HCB) and mirex in adipose tissue. The study is part of this laboratory's overall responsibility of selecting and testing methods for inclusion in both the Environmental Protection Agency's *Manual of Analytical Methods for Pesticides and Toxic Substances in Human and Environmental Media* (1) and the AOAC *Official Methods of Analysis* (2). A collaborated procedure for HCB and mirex

was deemed a particularly necessary adjunct to the EPA manual's modified Mills, Onley, and Gaither method for chlorinated hydrocarbon pesticides and metabolites in human tissue and excreta (1). Neither compound is quantitatively recovered by the Mills, Onley, and Gaither procedure for nonpolar residues in human tissues. An unfavorable partitioning ratio in the petroleum ether-acetonitrile partition cleanup accounts for low recoveries of both.

We did not evaluate the procedure collaboratively studied by Bong (3) for hexachlorobenzene and mirex in butterfat and fish because of sample size restrictions in human adipose and lack of a confirmation scheme. The procedure of Crist et al. (4) was chosen for study because it offered both improved recoveries and an HCB confirmatory derivative analysis. The confirmation scheme is especially useful for HCB because it elutes very early from gas chromatographic (GC) columns commonly used for pesticide residue analysis (1). Although the Crist et al. procedure was developed specifically for determination of HCB residues, a laboratory investigation also demonstrated its usefulness for mirex residues in adipose tissue.

The Crist et al. method features direct application of rendered adipose tissue to a Florisil column and elution with hexane. Mirex and HCB are determined by direct GC of the concentrated eluate. HCB residues are then confirmed by GC determination of an ether derivative formed by reaction with ethanol, 1-propanol, 2-propanol, or butanol. Mirex residues do not survive this reaction.

The procedure was subjected to an exhaustive intralaboratory examination before formal interlaboratory collaborative study. Upon our

evaluation of the Crist et al. method, the reaction with 2-propanol to form the disubstituted bis-isopropoxytetrachlorobenzene (BITB) product was determined to be the most suitable procedure. After minor modification, the procedure was collaboratively studied by 12 laboratories.

Collaborative Study

Each participating laboratory was presented with a package containing a copy of the paper by Crist et al. (4); specific technical instructions including method modifications; pre-tested Florisil; pyridine (determined to be free of electron-capturing interferences); individual HCB and mirex known standard solutions in isooctane; an unknown standard mixture of HCB and mirex; 5 g rendered chicken fat blank; 9 unknown rendered chicken fat samples fortified with both HCB and mirex; and a report sheet for results of the unknowns and sample background values for HCB and mirex. Collaborators were asked to perform trial runs on rendered fat blanks fortified with HCB and mirex at 20–50 and 100–500 ppb, respectively. Any method difficulties were to be resolved by the collaborator and/or coordinating laboratory before proceeding with the unknowns.

Hexachlorobenzene (HCB) and Mirex in Adipose Tissue—Official First Action

(Caution: See 51.037, 51.041, 51.061, and 51.072.)

29.A01 Reagents

(a) *Hexane, 2-propanol*.—See solv. purity test, 29.002.

(b) *Sodium sulfate*.—Anhyd. granular, Soxhlet-extd with hexane.

(c) *Sodium sulfate*.—2% aq. soln.

(d) *Florisil column*.—Packed with 100 mm Florisil and 12 mm Na_2SO_4 on top. Hold in $130 \pm 2^\circ$ oven ≥ 16 hr prior to use. (Remove stopcocks.) Prewash with 50 mL hexane immediately before use. Predetermine HCB and mirex elution on each lot of Florisil.

(e) *"Keeper" soln*.—1% paraffin oil in hexane.

(f) *Pyridine*.—Burdick and Jackson, or equiv., suitable for liq. and gas chromatg. Check pyridine in a reagent blank before use.

(g) *Potassium hydroxide soln*.—10% KOH in 2-propanol.

29.A02 Apparatus

(a) *Gas chromatograph*.—With ^3H or ^{63}Ni electron capture detector and 1.8 m $\times$ 4 mm id borosilicate glass columns packed with 1.5% OV-

17/1.95% OV-210 or 5% OV-210 on 80–100 mesh Gas-Chrom Q. *Typical operating conditions*.—Carrier gas flow rate 60 mL/min, column temp. 200° (OV-17/OV-210); 180° (5% OV-210).

(b) *Glassware*.—300 $\times$ 25 mm od Chromoflex column for Florisil (Kontes, K-420530), Kuderna-Danish (K-D) concentrator assembly (K-570000) fitted with 25 mL graduated tube (K-570050, size 2525, 19/22, 25 mL), modified micro Snyder column (K-569250), disposable pipets.

29.A03

Determination

Accurately weigh ca 0.5 g rendered or extd fat in tube. Dissolve fat in ca 0.5 mL hexane and transfer quant. to Florisil column pre-washed with hexane. Rinse tube with two 0.5 mL portions hexane. Let column elute until solv. level is just at top of Na_2SO_4 . Rinse column sides with 2–3 mL hexane. Elute with 200 mL hexane at 5 mL/min.

Collect eluate in K-D flask equipped with 25 mL concentrator tube. Evap. on steam bath to ≥ 10 mL. Use warm (50°) H_2O bath and gentle N stream for further vol. reduction.

Inject 3–8 μL stds and samples into OV-17/OV-210 column. Alternatively, OV-210 column at 180° may be used for mirex quantitation. Adjust sample vol. as required to produce major peak responses. Peak hts of stds and samples should not vary $>25\%$. Do not quantitate $<20\%$ full scale deflection. Amts injected must fall within linear range of detector. Work at same attenuation.

29.A04

Confirmation of HCB

Prep. sample for derivatization by evapg hexane ext to 0.1–0.2 mL with warm H_2O bath and gentle N stream. Derivatize ≥ 3 stds along with samples (HCB stds must bracket HCB concn in samples as detd by initial GLC analysis. Response of HCB stds must be linear.) Add 2–3 drops 1% paraffin oil in hexane to stds as "keeper" before evapg to 0.1–0.2 mL.

Add 0.5 mL 10% KOH in 2-propanol and 0.2 mL pyridine to each sample and std. Gently tap tube to mix. Place modified micro Snyder columns on concentrator tubes and place tubes in boiling H_2O bath 45 min. Remove tubes and cool under cold tap H_2O . Add ca 10 mL 2% Na_2SO_4 soln to each tube and exactly 2 mL hexane. Mix vigorously 1 min. Let solv. phases sep. completely. Inject 3–8 μL hexane phase into gas chromatograph for quantitation. Adjust hexane vol. as required. Calc. concn HCB. Mirex will not be recovered thru derivative procedure.

Results and Discussion

The 9 fortified fat unknowns consisted of 3 repetitions of 3 spiking levels. Samples 1, 5, and 8 were identical and represented 20.0 and 150 ppb fortifications of HCB and mirex, re-

Table 1. Individual laboratory results (ppb) for HCB, mirex, and HCB derivative in adipose tissue

Lab.	Fat blank	Sample								
		1	2	3	4	5	6	7	8	9
Hexachlorobenzene										
1	2.8	21.0	32.0	59.0	48.0	23.0	34.0	44.0	22.0	28.0
2	2.95	20.8	30.2	40.6	60.6	21.3	31.5	54.3	21.5	45.9
3	2.05	21.0	30.3	50.5	50.4	18.6	32.0	48.0	22.6	34.6
4	2	16.0	28.0	66.0	60.0	25.0	33.0	24.0	27.0	36.0
5	2.2	19.2	32.0	48.0	52.0	19.6	33.0	54.6	20.0	28.0
6	1.45	16.6	31.5	39.0	38.0	18.3	30.1	39.6	20.8	29.2
7	5.58	19.2	30.7	39.8	44.3	20.6	29.7	42.9	12.0	28.7
8	2.7	21.0	29.0	45.0	50.0	24.0	34.0	44.0	20.0	32.0
9	7	40.6	46.4	63.7	40.3	19.3	36.4	62.5	26.5	39.3
10	3.02	17.2	25.9	40.1	34.7	22.6	28.2	29.7	13.7	29.5
11	3.7	24.8	37.6	47.1	48.0	20.0	32.8	48.1	21.5	31.7
12	0	21.2	31.4	54.6	54.2	22.9	34.4	52.5	20.7	32.6
Mirex										
1	10	144	296	468	552	146	288	449	154	277
2	32.3	165	272	469	596	161	292	504	171	387
3	15.7	164	319	590	551	193	362	563	173	343
4	20	133	180	447	400	176	216	200	172	240
5	8	156	288	498	517	154	307	507	157	274
6	— ^a	121	221	322	282	122	209	334	132	198
7	6.26	138	312	452	540	157	281	521	139	302
8	55	197	332	522	589	185	360	558	165	345
9	96.3	173	377	679	482	150	14 633 ^b	584	183	376
10	13.6	149	297	489	403	198	268	334	107	293
11	—	150	301	485	483	146	296	488	152	294
12	—	159	312	608	564	158	320	562	176	312
HCB Derivative										
1	3.0	20.0	29.0	32.0	31.0	20.0	20.0	48.0	18.0	30.0
2	—	—	—	—	—	—	—	—	—	—
3	2.0	18.3	38.7	21.7	52.4	21.2	11.2	42.1	8.0	10.0
4	—	—	—	—	—	—	—	—	—	—
5	4	20.0	41.0	56.0	56.0	20.0	29.0	47.0	24.0	32.0
6	—	15.3	29.0	33.7	35.9	18.1	26.9	37.4	19.7	24.0
7	7.5	24.8	36.2	40.1	50.9	26.0	28.8	43.5	15.6	34.4
8	0	10.0	17.0	21.0	39.0	18.0	36.0	47.0	23.0	39.0
9	0	7.5	5.7	4.5	14.8	5.3	5.2	8.7	0.0	10.2
10	2.86	13.3	21.0	37.9	25.6	17.0	20.5	30.3	13.7	28.3
11	3.9	24.0	37.8	51.6	53.7	20.3	36.0	53.2	19.3	34.5
12	—	15.2	26.1	34.0	36.0	13.6	28.2	43.2	16.0	25.9

^a Not reported.^b Outlier; sample blank problem.

spectively. Samples 2, 6, and 9 were identical and represented 33.3 and 300 ppb fortifications of HCB and mirex, respectively. Samples 3, 4, and 7 were identical and represented 50.0 and 500 ppb fortifications of HCB and mirex, respectively. Analysts were specifically requested to determine HCB and mirex blank values but not to subtract them from their results. The unknown standard solution contained 8 pg HCB and 96 pg mirex/ μ L. The analysis of this standard solution was a measure of the performance of the GC instrumentation and the analyst's quantitation technique since the provided known standards

were to be merely diluted and compared directly by GC with the unknown solution.

Table 1 is a compilation of the results reported by each laboratory. Sample numbers 1, 5, 8; 2, 6, 9; and 3, 4, 7 are the low, middle, and high level fortifications for both compounds, respectively. Certain collaborators did not report fat blank values for the mirex and HCB derivative determination.

Tables 2-4 present a summary of the collaborative results for the unknown standard solutions, fat blank, HCB before and after derivatization, and mirex. Each value reported for fortified fat represents an average of the

Table 2. HCB collaborative results

Lab.	Unknown std (8 pg/ μ L) and %	Fat blank, ppb	Fat fortification, ppb						Av. intralab. CV, %
			Av. ppb, and std dev.			Intralab. CV, %			
			20.0	33.3	50.0	20.0	33.3	50.0	
1	8.0 ^a 100.0	2.80	22.0 1.00	31.3 3.06	50.3 7.77	4.5	9.8	15.4	9.9
2	7.44 93.0	2.95	21.2 0.29	35.9 8.72	51.9 10.22	1.4	24.3	19.7	15.1
3	7.75 96.9	2.05	20.7 2.01	32.3 2.17	49.6 1.42	9.7	6.7	2.9	6.4
4	12.5 ^a 156.3	2.00	22.7 5.85	32.3 4.04	50.0 22.72	25.8	12.5	45.4	27.9
5	8.0 100.0	2.20	19.60 0.40	31.0 2.65	51.5 3.32	2.0	8.5	6.4	5.6
6	7.5 93.8	1.45	18.6 2.11	30.3 1.15	38.9 0.81	11.3	3.8	2.1	5.7
7	7.28 91.0	5.58	17.3 4.62	29.7 1.03	42.4 2.31	26.7	3.5	5.4	11.9
8	7.7 96.3	2.70	21.7 2.08	31.7 2.52	46.3 3.21	9.6	7.9	6.9	8.1
9	9.3 116.3	7.0 ^a	28.8 ^a 10.83	40.7 ^a 5.14	55.5 13.18	37.6	12.6	23.7	24.6
10	7.91 98.9	3.02	17.8 4.48	27.9 1.82	34.8 ^a 5.20	25.2	6.5	14.9	15.5
11	9.0 112.5	3.70	22.1 2.46	34.0 3.14	47.7 0.55	11.1	9.2	1.2	7.2
12	7.6 95.0	0	21.6 1.15	32.8 1.51	53.8 1.12	5.3	4.6	2.1	4.0
Mean	7.95	2.58	20.5	31.7	48.9				
Mean % rec.	99.4		89.6 ^b	87.4 ^b	92.6 ^b				
Std dev.	0.64		1.86	2.14	4.87				
CV, %	8.05		9.1	6.8	9.96				

^a Outlier.^b Mean corrected for blank.

3 repetitive determinations with the standard deviation of the 3 values directly underneath. Using these figures, the percent intralaboratory coefficient of variation (CV) for each spiking level and the average intralaboratory CV value are calculated and reported on the same horizontal line of the table. The interlaboratory results are given at the bottom of each column. Outlier values were determined by the fitness test method described in the EPA Quality Control Manual (5).

Table 2 gives the HCB results for the unknown standard solution, fat blank, and the spiked fat samples analyzed before derivatization. The standard solution average result of 7.95 pg/ μ L represents a GC comparative accuracy of 99.4%. The 3 HCB sample results when corrected for the average fat blank of 2.58 ppb yielded interlaboratory average recovery values of 89.6, 87.4, and 92.6%, respectively, for the 20.0, 33.3, and 50.0 ppb fortifications. The respective interlaboratory CV values of 9.1, 6.8, and 9.96% demonstrate

excellent precision. The 3 outlier values for laboratory 9 are possibly explained by the author's comment that the Florisil column was not pre-wetted with hexane before sample elution.

Table 3 shows the HCB confirmatory analyses by formation of the bis-isopropoxytetrachlorobenzene (BITB) derivative. The 3 HCB interlaboratory average results when corrected for the fat blank of 2.25 ppb yielded percent recoveries of 79.8, 78.8, and 76.9, respectively, for the 20.0, 33.3, and 50.0 ppb fortifications. The respective interlaboratory CV values of 15.7, 18.6, and 19.0% demonstrate acceptable precision although understandably not nearly as good as the direct HCB determinations. Laboratory 9 again showed 3 outlier values, presumably for the same reason as mentioned above.

The Table 4 results for mirex indicate no difficulty with the unknown standard solution or the 3 fortification levels. The standard solution average of 97.5 pg/ μ L represents an

Table 3. HCB derivative collaborative results

Lab.	Fat blank, ppb	Fat fortification, ppb						Av. intralab. CV, %
		Av., ppb, and std dev.			Intralab. CV, %			
		20.0	33.3	50.0	20.0	33.3	50.0	
1	3.0	19.3 1.15	26.3 5.51	37.0 9.54	6.0	21.0	25.8	17.6
2	—	—	—	—				
3	2.0	15.8 6.9	20.0 16.2	38.7 15.6	43.7	81.0	40.3	55.0
4	—	—	—	—				
5	4.0	21.3 2.31	34.0 6.24	53.0 5.2	10.8	18.4	9.8	13.0
6	—	17.7 2.2	26.6 2.5	35.7 1.9	12.4	9.4	5.3	9.0
7	7.54 ^a	22.1 5.7	33.1 3.9	44.8 5.5	25.8	11.8	12.3	16.6
8	0	17.0 6.6	30.7 11.9	35.7 13.3	38.8	38.8	37.3	38.3
9	0	4.3 ^a 3.9	7.0 ^a 2.8	9.3 ^a 5.2	90.7	40.0	55.9	62.2
10	2.86	14.7 2.0	23.3 4.4	31.3 6.2	13.6	18.9	19.8	17.4
11	3.9	21.2 2.5	36.1 1.7	52.8 1.1	11.8	4.7	2.1	6.2
12	—	14.9 1.2	26.7 1.3	37.7 4.8	8.1	4.9	12.7	8.6
Mean	2.25	18.2	28.5	40.7				
Mean % rec.		79.8 ^b	78.8 ^b	76.9 ^b				
Std dev.		2.86	5.30	7.74				
CV, %		15.7	18.6	19.0				

^a Outliers.^b Mean corrected for blank.

average comparison of 101.6%. The 3 mirex average results, when corrected for the average blank of 27.5 ppb, yielded interlaboratory percent recovery values of 89.0, 90.2, and 92.3, respectively, for the 150, 300, and 500 ppb fortification levels. The respective interlaboratory CV values of 7.6, 16.5, and 18.1% represent excellent to acceptable precision values.

Table 5 is a display of the rank of averages for all results of fortified fat samples. Rankings of 1 to 12 correspond to the lowest to the highest values obtained by the 12 participants. Laboratory 9 was again noticeable with its extremes of both high and low values. Laboratory 10 also demonstrated a preponderance of low values. This latter laboratory was one of the last to complete the study and results might indicate concentrated standard solutions due to solvent evaporation.

Collaborator Comments

Some experimental difficulties were encountered by a few collaborators with the HCB derivative quantitations. The following

oral and/or written comments were received:

Laboratory 1 reported variability of results due to erratic formation of both mono- and disubstituted derivative products.

Laboratory 2 did not report HCB derivative results. The collaborator discussed problems with the authors by telephone, described erratic formation of significant quantities of both mono- and disubstituted reaction products, and therefore could not perform the quantitative confirmation analyses.

Laboratory 4 indicated the derivative analyses were not attempted.

Laboratory 5 indicated some difficulty with erratic derivative results and suggested that fresh base solution may permit more consistent results.

Reaction conditions were intended to ensure production of essentially all disubstituted reaction product. In summary, however, at least a few collaborators had some difficulty with the formation of the mono- as well as the disubstituted product. Table 2 intralaboratory CV values further demonstrate some precision difficulties.

Table 4. Mirex collaborative results

Lab.	Unknown std (% pg/ μ L) and %	Fat blank, ppb	Fat fortification, ppb						Av. intralab. CV, %
			Av. ppb, and std dev.			Intralab. CV, %			
			150	300	500	150	300	500	
1	89.8 93.5	10.0	148 5.29	287 9.54	490 54.8	3.6	3.3	11.2	6.0
2	96.2 100.2	32.3	166 5.10	317 61.0	523 66.0	3.1	19.2	12.6	11.6
3	92.0 95.8	15.7	177 14.8	341 21.6	568 20.0	8.4	6.3	3.5	6.1
4	103.0 107.3	20.0	160 23.8	212 30.2	349 131	14.9	14.2	37.5	22.2
5	96.0 100.0	8.0	156 1.53	290 16.6	507 9.50	1.0	5.7	1.9	2.9
6	97.6 101.7	—	125 ^a 6.03	209 11.5	313 27.2	4.8	5.5	8.7	6.3
7	101 105.2	6.26	145 10.8	298 15.6	504 46.5	7.4	5.2	9.2	7.3
8	100.0 104.2	55.0	182 16.2	346 14.0	555 33.5	8.9	4.0	6.0	6.3
9	99.2 103.3	86.3	169 17	377 1.27	582 98.6	10.1	0.34	16.9	9.1
10	101 105.2	13.6	151 45.5	286 15.7	409 77.7	30.1	5.5	19.0	18.2
11	97.2 101.3	—	149 3.06	297 3.61	485 2.52	2.1	1.2	0.52	1.3
12	84.7 ^a 88.2	—	164 10.1	315 4.6	578 26.0	6.2	1.5	4.5	4.1
Mean	97.5	27.5	161	298	489				
Mean % rec.	101.6		89.0 ^b	90.2 ^b	92.3 ^b				
Std dev.	3.97		12.2	49.3	88.3				
CV, %	4.1		7.6	16.5	18.1				

^a Outlier.^b Mean corrected for blank.

Conclusions and Recommendations

Satisfactory recoveries were obtained by the direct analysis procedure for HCB and mirex from fortified fat samples. The derivative pro-

cedure for HCB provides a qualitative and semiquantitative confirmation procedure.

It is recommended that the method be adopted official first action for direct determination of HCB and mirex and for qualitative and semiquantitative derivative confirmation of HCB.

Acknowledgments

The authors express their sincere thanks to the following study participants: Ana Barquet, Henry Enos, and Carmen Morgade, University of Miami, Perrine, FL; Jimmie Cain, Mississippi State Chemical Laboratory, Mississippi State, MS; James Colcolough, Jr, Medical University of South Carolina, Sullivan's Island, SC; Vaughn Cox and Edward Gomes, Texas Tech University School of Medicine, San Benito, TX; A. B. Filonow and Richard A.

Table 5. Rank of averages^a

Lab.	Fortification, ppb								
	HCB			HCB deriv.			Mirex		
	20.0	33.3	50.0	20.0	33.3	50.0	150	300	500
1	9	5	8	7	4	5	3	4	5
2	6	11	10	—	—	—	9	9	8
3	5	7	6	4	2	7	11	10	10
4	11	8	7	—	—	—	7	2	2
5	4	4	9	9	9	10	6	5	7
6	3	3	2	6	5	3.5	1	1	1
7	1	2	3	10	8	8	2	7	6
8	8	6	4	5	7	3.5	12	11	9
9	12	12	12	1	1	1	10	12	12
10	2	1	1	2	3	2	5	3	3
11	10	10	5	8	10	9	4	6	4
12	7	9	11	3	6	6	8	8	11

^a A rank of 1 indicates lowest of 12 values and a rank of 12 the highest of 12 values.

The recommendation of the authors was approved by the General Referee and Subcommittee E and was adopted by the Association. See *J. Assoc. Off. Anal. Chem.* (1980) 63, 313.

Leavitt, Michigan State University, East Lansing, MI; Brigita Gardner and Robert Stringer, Arkansas Department of Health, Little Rock, AR; Herbert Hetzler and Edward Slach, University of Iowa, Epidemiology Studies Program, Ames, IA; Anne-Marie C. Leventis, South Carolina Department of Health and Environment Control, Columbia, SC; Robert Morgner and Ernest Richardson, Maine Department of Health and Welfare, Augusta, ME; John Tessari, Colorado State University, Ft. Collins, CO; Stephen L. Warnick, Intermountain Laboratories, Salt Lake City, Utah; Robert L. Welch, Michigan Department of Public Health, East Lansing, MI. A special thanks is also extended to David Svensgaard

of EPA, Research Triangle Park, NC, for statistical analysis of data.

REFERENCES

- (1) *Manual of Analytical Methods for Pesticides and Toxic Substances in Human and Environmental Media* (1977), Environmental Protection Agency, Quality Assurance Section (MD-69), Research Triangle Park, NC 27711
- (2) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA
- (3) Bong, R. L. (1975) *J. Assoc. Off. Anal. Chem.* **58**, 557-561
- (4) Crist, H. L., Moseman, R. F., & Noneman, J. W. (1975) *Bull. Environ. Contam. Toxicol.* **14**, 273-279
- (5) *Manual of Analytical Quality Control for Pesticides in Human and Environmental Media* (1979) Watts, R. R., & Thompson, J. F., Environmental Protection Agency, Quality Assurance (MD-69), Research Triangle Park, NC 27711.

This paper was presented at the 93rd Annual Meeting of the AOAC, Oct. 15-18, 1979.

Received September 13, 1979. Accepted April 2, 1980.
